



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference NAE877 03PCT	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/EP2004/000099	International filing date (day/month/year) 09 January 2004 (09.01.2004)	Priority date (day/month/year) 09 January 2003 (09.01.2003)
International Patent Classification (IPC) or national classification and IPC C12N 1/15, 15/80, C12P 23/00, A23J 1/00, 3/00, A23L 1/28, 1/275		
Applicant BASF AKTIENGESELLSCHAFT		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

3. This report is also accompanied by ANNEXES, comprising:

a. (*sent to the applicant and to the International Bureau*) a total of 8 sheets, as follows:

sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).

sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

b. (*sent to the International Bureau only*) a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

<input checked="" type="checkbox"/>	Box No. I	Basis of the report
<input type="checkbox"/>	Box No. II	Priority
<input checked="" type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input checked="" type="checkbox"/>	Box No. IV	Lack of unity of invention
<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input checked="" type="checkbox"/>	Box No. VI	Certain documents cited
<input type="checkbox"/>	Box No. VII	Certain defects in the international application
<input type="checkbox"/>	Box No. VIII	Certain observations on the international application

Date of submission of the demand 15 July 2004 (15.07.2004)	Date of completion of this report 20 April 2005 (20.04.2005)
Name and mailing address of the IPEA/EP	Authorized officer
Facsimile No.	Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

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Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

This report is based on translations from the original language into the following language _____, which is language of a translation furnished for the purpose of:

- international search (under Rules 12.3 and 23.1(b))
- publication of the international application (under Rule 12.4)
- international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed"* and are not annexed to this report):

The international application as originally filed/furnished

the description:

pages _____ 1-93 _____, as originally filed/furnished
 pages* _____ received by this Authority on _____
 pages* _____ received by this Authority on _____

the claims:

pages _____, as originally filed/furnished
 pages* _____, as amended (together with any statement) under Article 19
 pages* 1-53 _____ received by this Authority on 16 March 2005 (16.03.2005)
 pages* _____ received by this Authority on _____

the drawings:

pages 1/24-24/24 _____, as originally filed/furnished
 pages* _____ received by this Authority on _____
 pages* _____ received by this Authority on _____

a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. The amendments have resulted in the cancellation of:

- the description, pages _____
- the claims, Nos. _____
- the drawings, sheets/figs _____
- the sequence listing (*specify*): _____
- any table(s) related to sequence listing (*specify*): _____

4. This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- the description, pages _____
- the claims, Nos. _____
- the drawings, sheets/figs _____
- the sequence listing (*specify*): _____
- any table(s) related to sequence listing (*specify*): _____

* If item 4 applies, some or all of those sheets may be marked "superseded."

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Box No. III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- the entire international application
- claims, Nos. _____

because:

- the said international application, or said claims Nos. _____

relate to the following subject matter which does not require an international preliminary examination (*specify*):

- the description, claims or drawings (*indicate particular elements below*) or said claims Nos. _____
are so unclear that no meaningful opinion could be formed (*specify*):

- the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed (*specify*):

- no international search report has been established for said claims Nos. _____
- a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:
 - furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
 - furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
 - pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13ter.1(a) or (b) and 13ter.2.
- a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.
- See Supplemental Box for further details.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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PCT/EP2004/000099**Box No. IV Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has, within the applicable time limit:
 - restricted the claims
 - paid additional fees
 - paid additional fees under protest and, where applicable, the protest fee
 - paid additional fees under protest but the applicable protest fee was not paid
 - neither restricted the claims nor paid additional fees
2. This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:
 - complied with
 - not complied with for the following reasons:
4. Consequently, this report has been established in respect of the following parts of the international application:
 - all parts
 - the parts relating to claims Nos. _____

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	
1. Statement		
Novelty (N)	Claims	<u>2, 8, 10-11, 13, 15-18</u> YES
	Claims	<u>1, 3-7, 9, 12, 14, 19, 20, 21</u> NO
Inventive step (IS)	Claims	_____ YES
	Claims	<u>1-21</u> NO
Industrial applicability (IA)	Claims	<u>1-21</u> YES
	Claims	_____ NO

2. Citations and explanations (Rule 70.7)Subject matter of the present application

A method for producing carotenoids, involving (a) the preparation of genetically engineered organisms of the *Blakeslea* genus produced by transformation, (b) cultivation of the cells, and (c) preparation of the produced carotenoid.

The preparation of genetically engineered organisms of the *Blakeslea* genus produced by transformation is subject matter of the application EP04/00100.

Cited documents (PCT Rule 64.1)

- D1: WO 98/46772 A
- D2: US-B1-6 413 736
- D3: US 2002/051998 A1
- D4: LEE ET AL. (2002) APPL. MICROBIOL. BIOTECHNOL. 60, 1-11
- D5: WO 98/03480 A
- D6: WO 98/50574 A

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International application No.

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Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
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Novelty (PCT Article 33(2))

The present application does not meet the requirements of PCT Article 33(1) because the subject matter of claims 1, 3 to 7, 9, 12, 14, 19, 20 and 21 lacks novelty (PCT Article 33(2)).

Document D1 is considered the prior art closest to the subject matter of claim 1 and discloses the production of genetically transformed filamentous fungi and fungi of the *Blakeslea* genus (page 16, line 6). The intention is to use the produced fungi for producing secondary metabolites and carotenoids (page 27, line 9). It is also the intention to carry out genetic engineering of the genome, for example by homologous recombination. D1 discloses (page 19, lines 5 to 29) that if the genetically engineered fungi are intended for the production of secondary metabolites, then expression cassettes with the requisite genes can be introduced into the genome. The selection and propagation of the genetically engineered cells are disclosed, for example, in claims 21 and 22. A method for producing metabolites is disclosed in claim 28.

D1 is considered prejudicial to novelty, since the examiner is of the opinion that the information in D1 matches the imprecise and broad wording of the present claims.

Inventive step (PCT Article 33(3))

The present application does not meet the requirements of

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

PCT Article 33(1) because the subject matter of claims 1 to 38 does not involve an inventive step (PCT Article 33(3)).

The subject matter of claim 2 differs from that known from D1 in that the method is restricted to fungi of the *Blakeslea trispora* type.

The present invention can thus be considered to address the problem of developing a method for producing genetically engineered *Blakeslea trispora*.

In view of the fact that

- claim 1 does not contain any special features relating to the transformation,
- D1 discloses the production of genetically engineered fungi of the *Blakeslea* genus,
- organisms of the *Blakeslea trispora* type are known for their production of β -carotene, and
- genetic mutations for increasing carotenoid production of the *Blakeslea trispora* type are known,

an inventive step cannot be acknowledged in respect of claim 2.

The use of a plasmid containing a gpdA promoter, hph resistance gene and/or trpC terminator for transformation purposes is also not inventive, since it is known to a person skilled in the art (see also the international preliminary examination report relating to PCT application EP04/00100).

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

As has already been mentioned in the description of the present application, homokaryotisation is necessary only when a genomic gene deletion is intended to be carried out, not, however, when genes are intended to be added, and is the intention in D1. As an alternative to homokaryotisation, it would also be possible, however, to carry out RNA silencing.

D2, D3 and D4 disclose the genetic production of carotenoids and xanthophylls, and transformed organisms which contain the genes required therefor. It is obvious to use this knowledge to genetically produce organisms of the *Blakeslea* genus, since it is known that said organisms produce large quantities of β -carotene.

The vector with SEQ ID NO:3 is derived from the known vector pANsCOS1, which was developed for the transformation of fungi (Osiewacz (1994) Curr. Genet. 26, 87-90). The use of the vector with SEQ ID NO:3 and the vectors derived therefrom in a method for the transformation of *Blakeslea* is therefore non-inventive.

Industrial applicability (PCT Article 33(4))

The industrial applicability of claims 1 to 22 and 50 to 52 (in part) is acknowledged.

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Box No. VI Certain documents cited**1. Certain published documents (Rule 70.10)**

Application No. Patent No.	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
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2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure (day/month/year)	Date of written disclosure referring to non-written disclosure (day/month/year)
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See Supplemental Sheet

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

I Amendments (PCT Article 34(2)b, PCT Rule 70.2)

The amendments submitted with the letter of 16 March 2005 do not introduce substantive matter which goes beyond the disclosure in the international application.

IV Unity of invention (PCT Rule 13)

The International Searching Authority (ISA) determined that the originally filed version of the present application did not meet the requirements of PCT Rule 13.

The originally filed independent method claims 1, 22 and 35 and product claims 48, 49 and 53 of the present application are linked by the feature "carotenoids".

It has long been known that organisms of the *Blakeslea* genus produce carotenoids. It has also long been known that the carotenoids produced by organisms of the *Blakeslea* genus can be used as foodstuffs or as nutritional supplements (see the description, WO-98/03480 and WO-98/50574). Furthermore, the carotenoids, foodstuffs and nutritional supplements obtainable using the method cannot be distinguished from those which are produced using other methods that are known *per se*.

The originally filed method claims and product claims were therefore not linked by a single novel feature and therefore did not satisfy PCT Rule 13.

Supplemental Box

The originally filed substantive matter was therefore divided into the following three groups:

- A) method of preparing carotenoids or precursors thereof by means of genetically modified organisms of the *Blakeslea* genus (claims 1 to 21);
- B) method of providing carotenoids, foodstuffs and nutritional supplements (claims 22 to 47 and 50 to 52 (in part)); and
- C) carotenoids, foodstuffs and nutritional supplements produced according to the method as per invention A (claims 48, 49, 53 and 50-52 (in part)).

The applicant was requested to pay additional search fees. The applicant decided to neither pay additional search fees nor to lodge an objection. The international search report was therefore restricted to invention A (originally filed claims 1 to 21).

With the letter of 16 March 2005, the applicant restricted the substantive matter in group B to the method in group A. The present independent method claims 1, 22 and 35 therefore have the method as per claim 1 as the linking feature.

The present and originally filed claims 1 to 21 disclose features which relate to genetically engineered organisms of the *Blakeslea* genus. In contrast, the present and originally filed claims 22 to 47 disclose features which relate to the purification of the produced carotenoids.

Supplemental Box

Since the examiner is of the opinion that claim 1 is not novel and inventive in relation to D1, independent method claims 1, 22 and 35 are not linked by a novel and inventive feature.

The objection concerning a lack of unity of invention is therefore upheld.

III No preliminary examination

Since the examiner is of the opinion that:

- 1) the feature linking method claims 1, 22 and 35 is not novel and inventive (see Box V), and
- 2) the international search report relates only to the features mentioned in the originally filed claims 1 to 21, that is to features of genetically engineered organisms, but not to features concerning the purification of carotenoids, no international preliminary examination report (IPER) can be established in respect of the subject matter of the present claims 22 to 47 (PCT Rule 66.1(e)).